EXPERIMENTAL FINDINGS OF THE TISSUE CULTURE OF ORCHID HYBRIDS AT THE SINGAPORE BOTANIC GARDENS

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ABSTRACT

A Tissue Culture Laboratory was established at the Singapore Botanic Gardens in the early 1970's. By 1975 several orchid hybrids had been successfully mericloned and in 1978 the Laboratory started a tissue culture service to assist local orchid growers and breeders. To date, the Laboratory has successfully mericloned six species and more than one hundred orchid hybrids. Some effective media compositions have been established and used in research and routine commercial production at the Laboratory. Tissue culture techniques using leaves and lateral buds of flower stalks have also been employed. Various tissue culture techniques and media used for local orchids is presented.

INTRODUCTION

Research work on orchids at the Botanic Gardens, Singapore started in 1928 and early work was mainly on hybridisation. Following the success in tissue culture of orchids by G.M. Morel (1960) in France, it was decided to venture into this new line of research at the Botanic Gardens. A Tissue Culture Laboratory was established in the early 1970's and preliminary experiments were conducted with improvised equipment. In 1973 one laminar flow and two shakers were acquired, however these shakers operated on a pulley system which broke down frequently. In 1975 a Brunswick shaker was purchased. By that time, several hybrids of *Dendrobium* (Singh, 1976), *Aranda, Aranthera,* and *Burkillara,* had been successfully mericloned. After many attempts, *Dendrobium attennatum* was also successfully mericloned in 1977.

In late 1978 the Tissue Culture Laboratory started a mericlone service to assist local orchid growers and breeders. The Laboratory undertakes experimental tissue culture propagation of parent plants sent in by the public. When fully differentiated plantlets are obtained the experiment is considered successful and a contract is then agreed upon between the owner of the parent plants and the Laboratory for the purchase and supply of a certain number of plantlets. Plantlets supplied are at least 3 cm in length with strong, healthy roots and in a condition ready for potting. Orchid hybrids which have been mericloned at the Laboratory and supplied commercially on a large scale include *Dendrobium* Tay Swee Keng, *Dendrobium* Yong Kok Wah, *Dendrobium* Jaquelyn Hawaii, *Aranda* Chong Chee Yuen, *Kagawara* Teoline Fair, *Mokara* Sally Lim, and *Aranda* Ng Chong Hang. Facing keen competition from Thailand and Malaysia on the international market for cut flowers, Singapore orchid growers and breeders have increasingly sought the assistance of the Tissue Culture Laboratory for the mass propagation of their choice hybrids. The Laboratory has, therefore, been able to contribute directly to the rapid growth, both in terms of the total annual export and the level of technical sophistication, of the local orchid industry by making readily available an efficient mass propagation technique based on advanced biological knowledge.

Orchid Tissue Culture Techniques

In the poineering work of Morel (1960) shoot apices of *Cymbidium* were excised to produce virus-free protocorms which later developed into plantlets. The techniques used at the Tissue Culture Laboratory are basically variations of Morel's methods. Young leaves (Fu, 1979), axillary buds, lateral buds of flower stalks (Fu, 1978), and roots are sometimes used instead of apical shoots. Various media have been tested and hormones are occasionally added to the basic salt to induce protocorm formation, to accelerate growth, and to promote protocorm differentiation.

Plant Tissues Used

Using the shoot apex of an orchid plant for tissue culture requires the sacrifice of new growth or even, as in the case of monopodial orchids, the whole plant. Since success cannot be guaranteed and most of the plants selected for tissue culture are valuable (sometimes it is the only specimen of a new hybird of great commercial potential), the cost and risk involved could be high. It is, therefore, desirable to develop techniques using tissues which can be removed without doing much harm to the parent plants. Experiments have been conducted using young leaves, axillary buds, lateral buds of flower stalks, and roots as explants. Some of these experiments were successful and the methods developed have been adopted in routine production at the Tissue Culture Laboratory (see Tables 1 and 2). The parts taken from parent plants for the various tissue culture techniques are summarised below:

Culture technique	Part of plant used
Apical and axillary meristems	
sympodial orchids	young side shoot measuring 7–8 cm
monopodial orchids	top 10 cm of the apical shoot
Leaf	whole young leaf (3–4 x 1–2 cm) from mature plant
Lateral bud (flower stalk)	whole flower stalk about 20 cm in length
Root	young aerial root about 5 cm in length

Sterlization

Prior to excision, all plant parts must be sterilized. The methods for the various plant parts are as follows:

1. Apical and axillary meristems: The leaves are removed to expose the apical or axillary buds. The remaining shoot is then washed with antiseptic soap and soaked in a chlorox solution (10–12%) for 10–15 minutes.

2. Leaves: Cut leaves are washed with antiseptic soap and soaked in a chlorox solution (10–12%) for 15–20 minutes.

3. Lateral buds of flower stalks: Scale leaves are removed from the flower stalks to expose the lateral buds. The remaining stalk is then washed with antiseptic soap

and soaked in a chlorox solution (12–15%) for 20 minutes.

4. Roots: Cut aerial roots are washed with antiseptic soap and soaked in a chlorox solution (15%) for half an hour.

Excision

In meristem culture, 0.2 mm cubes are excised from sterilized plant parts and care is taken to excise only the meristematic cells and not the surrounding tissues. In root culture, young root tips 1–2 cm long are cut for use as explants. In leaf culture, whole young leaves are used.

Inoculation

All excised plants are put into 100 ml conical flasks continaing 25 ml of liquid media under sterilized conditions. These are then sealed and placed on shakers.

Shaking and environmental conditions

Shaking is essential during the early stages of protocorm formation (Wimber, 1963, 1965; Kunisaki *et al*, 1972). The basic purposes of shaking are to increase aeration, to reduce plant polarity, to enhance cell multiplication, to dilute excretion of explants, and to help distribute nutrients. From our experience, the mode of shaking is not significant however, too violent shaking has been observed to cause browning-off of protocorms.

In the Tissue Culture Laboratory, explants in shakers are exposed to continuous illumination of about 2000 lux and maintained at a temperature of 21–25°C.

Media

The most commonly used media for tissue culture of orchids are the White (1943) medium, the Murashige and Skoog (1962) medium, the Vacin and Went (1949) medium, and the Knudson (1949) C medium. These and many modified forms of these media have been tested at the Tissue Culture Laboratory. The most successful of the modified media are: KCBP medium – Knudson C basic salt supplemented with banana and pineapple juice; and VWM medium – Vacin and Went basic salt supplemented with banana, coconut milk, and tomato juice.

The media used depend on the stage of development of the protocorms. For protocorm initiaton, the Vacin and Went medium and the Knudson C medium have been found to be far superior to the others for apical shoot tip and axillary bud cultures. For most species and some hybrids it is necessary to add NAA or IAA to induce protocorm formation. In the case of tissue culture using leaves and lateral buds of flower stalks, the Murashige and Skoog medium supplemented with 2–4–D and 6–amino–benzyladenine has been found to be best for protocorm initiation.

For protocorm multiplication and differentiation, the Vacin and Went medium supplemented with coconut milk and sugar is best for most hybrids, especially for *Dendrobium* hybrids. *Vanda* hybrids, however, have been observed to multiply and differentiate faster in the Vacin and Went medium supplemented with coconut milk only. For most *Aranthera* hybrids, the best medium for multiplication and differentiation is the Murashige and Skoog basal salt.

The VWM medium has been found to be the best for the final stage of most hybrids. For some slow-growing hybrids such as *Aranda* Majula 'Rimau' and *Aranda* Christine No. 5, root formation is much faster in the KCBP medium than in the VWM medium. All *Oncidium* hybrids fail to form roots in the VWM medium, but satisfactory root formation is obtained in the KCBP medium. Since species of *Arachnis* and *Aranthera* hybrids excrete strong phenolic componds, it is necessary to change media every few days during the first six months of their growth. The composition of the media used in the Tissue Culture Laboratory is listed in Table 3. The appropriate media for some local species or hybrids at different stages of development are given in Tables 1 and 2.

Data and observations on tissue culture of local orchids

To date the Tissue Culture Laboratory has successfully mericloned 6 species and more than 110 hybrids of orchids. All the species are grown locally and out of the hybrids, 82 are local. Summaries of the basic data, part of plant used, media, growth rate, and relevant remarks on the tissue culture of these local orchid species and hybrids are given in Tables 1 and 2. These tables also show that many orchids have been successfully mericloned and it is possible that all orchid hybrids can be mericloned provided the right media is used.

Coconut milk appears to be an important supplement to all the media used in every stage of protocorm development. Coconut milk is believed to contain hormones (probably cytokinins) in a balanced composition (Letham, 1974) which are effective in inducing protocorm formation and differentiation. Auxins such as NAA and IAA are occasionally added to media to induce protocorm formation, however, they are found to have an inhibitory effect on the differentiation of protocorms.

A wide range variation in growth rates has been observed. Fast-growing hybrids produce marketable plantlets in about one year's time while slow-growing hybrids may take more than two years. Most *Dendrobium* hybrids have been found to be fast-growing and except for some *Aranda* hybrids, monopodial ground orchids are generally slow-growing. Experience seems to suggest, however, that the slow growth of many hybrids may probably be due to the use of unsuitable media rather than to some basic physiological characteristics of the hybrids. Considerable research effort in the Tissue Culture Laboratory has, therefore, been on the improvement of media in use and the search for effective new media.

Acknowledgement

The Tissue Culture Laboratory was developed due to the efforts of many researchers, especially Dr. Fu-Fan May Lay and Dr. Choo Yon Sen.

TABLE 1. LOCAL ORCHID SPECIES SUCCESSFULLY MERICLONED

	Source of meristem explant	Growth rate	Media for initiation (liquid)	Media for multiplication and differentiation	Media for plantlet formation	Remarks
Arachnis						
1. *hookerana*	apical & axillary	extremely slow	VWN + NAA	VWN, VWS	VWM	*Arachnis* spp. often excrete strong phenolic compounds; need to change medium weekly during the first six months
Dendrobium						
1. *antennatum*	apical	slow	VWS + NAA	VWN, VWS	VWM	
2. *laciniosum*	apical & axillary	slow	VWS	VWN, VWS	VWM	
3. *superbiens* 'Superba'	apical	fast	VWS + NAA	VWN, VWS	VWM	
Oncidium						
1. *lanceanum*	apical	slow	VWS	VWS	VWM	
2. *haematochilum*	apical	fast	VWS	VWN, VWS	KCMP	

TABLE 2. LOCAL ORCHID HYBRIDS SUCCESSFULLY MERICLONED

	Parentage	Source of meristem explant	Growth rate	Media for initiation (liquid)	Media for multiplication & differentiation	Media for plantlet formation	Remarks
Aeridachnis							
1. Bogor 'Apple Blossum'	*Arachnis hookerana x Aerides odorata*	apical & axillary	slow	VWN	VWN, VWS	VWM	
2. Alexandra	*Aeridachnis* Bogor x *Arachnis flosaeris* 'insignis'	apical & axillary	slow	VWN	VWN, VWS	VWM	
3. Elizabeth Howie	*Arachnis* Ishbel x *Aerides lawrenceae*	apical & axillary	slow	VWN	VWN, VWS	VWM	
Arachnis							
1. Capama 'merah'	*Arachnis* Maggie Oei x *Arachnis breviscapa*	apical & axillary	slow	VWN	VMN, VWS	VWM	
Arachnostylis							
1. Chorchalood	*Arachnis hookerana x Rhynochostylis gigantea*	apical & axillary	slow	VMW	VWN, VWS	VWM	
Aranda							
1. Belzonica	*Arachnis* Ishbel x *Vanda luzonica*	apical & axillary	slow	VWN	VWN, VWS, MS	VWM	MS medium best for differentiation
2. Bertha Braga	*Vanda tricolor* x *Arachnis* Maggie Oei	apical & axillary	slow	VWN	VWN, VWS	VWM	
3. Bintang	*Arachnis hookerana* x *Vanda* Tatzeri	axillary	fast	VWN/KC	VWN, VWS	VWM	
4. Chong Chee Yuen	*Aranda* Blue Star x *Vanda* Rothschildiana	axillary	fast	VWN/KC	VWN, VWS, MS	VWM/ KCBP	MS medium best for differentiation, root formation faster in KCBP than in VWM
5. Chen Chin Mu	*Aranda* Bintang Raffles x *Vanda* Wangse	axillary	fast	VWN/KC	VWN, VWS	VWM	
6. Christine No. 1	*Arachnis hookerana* x *Vanda* Hilo Blue	apical & axillary	fast	VWN/KC	VWN, VWS	VWM	
7. Christine No. 5	*Arachnis hookerana* x *Vanda* Hilo Blue	apical & axillary	slow	VWN	VWN, VWS	VWM/ KCBP	root formation faster in KCBP than in VWM
8. Christine No. 9	*Arachnis hookerana* x *Vanda* Hilo Blue	apical & axillary	fast	VWN	VWN, VWS	VWN	
9. Christine No. 27	*Arachnis hookerana* x *Vanda* Hilo Blue	whole leaf	slow	MS+2-4-D+ BA	VWN, VWS	VWM	
10. Christine No. 80	*Arachnis hookerana* x *Vanda* Hilo Blue	apical & axillary	fast	VWN	VWN, VWS	VWM	

TABLE 2. LOCAL ORCHID HYBRIDS SUCESSFULLY MERICLONED

	Parentage	Source of meristem explant	Growth rate	Media for initiation (liquid)	Media for multiplication & differentiation	Media for plantlet formation	Remarks
Aranda							
11. Christine No. 130	*Arachnis hookerana* x *Vanda* Hilo Blue	whole leaf	fast	MS+2-4-D +BA	VWN, VWS	VWM	
12. Christine No. 999	*Arachnis hookerana* x *Vanda* Hilo Blue	apical & axillary	fast	VWN	VWN, VWS	VWM	
13. Elizabeth Douglas Home	*Arachnis* Maggie Oei x *Vanda* Radman	apical & axillary	fast	VWN	VWN, VWS	VWM	
14. Eric Mekie	*Aranda* Lucy Laycock x *Vanda luzonica*	apical & axillary	fast	VWN	VWN, VWS	VWM	
15. Freckles	*Arachnis* Ishbel x *Vanda dearei*	apical & axillary	slow	VWN	VWN, VWS	VWM	
16. Hatifah	*Arachnis hookerana* x *Vanda luzonica*	axillary axillary	slow	VWN	VWN, VWS	VWM	
17. Hilda Galistan	*Arachnis hookerana* x *Vanda tricolor suavis*	apical & axillary	slow	VWN	VWN, VWS	VWM	
18. Imelda Romualdez Marcos	*Arachnis maingavi* x *Vanda* Harvest Time	apical & axillary	fast	VWN/KC	VWN, VWS	VWM	
19. Jessie	*Arachnis hookerana luteola* x *Vanda* Ellen Noa	apical & axillary	slow	VWN	VWN, VWS	VWM	
20. Lucy Laycock	*Arachnis hookerana* x *Vanda tricolor*	apical & axillary	slow	VWN	VWN, VWS	VWM	
21. Majula 'Rimau'	*Arachnis* Maggie Oei x *Vanda insignis*	axillary	slow	VWN	VWN, MS, VWS	KCBP/ VWM	MS medium is best for differentiation, root formation faster in KCBP than in VWM
22. Ng Chong Hang	*Aranda* Bintang Raffles x *Vanda* Rose Davis	apical & axillary	fast	VWN	VWN, VWS	VWM/ KCBP	
23. Noorah Alsagoff	*Arachnis hookerana* x *Vanda* Dawn Nishimura	leaf base	fast	MS+2-4-D+ BA	VWN, VWS	VWM	
24. Peter Ewart	*Arachnis hookerana* x *Vanda* Kapoho	axillary	fast	VWN	VWN, VWS	VWM	
25. Queen of Purples	*Arachnis hookerana* x *Vanda coerulea*	axillary & leaf base	fast	VWN/KC	VWN, VWS	VWM	
26, Tourism Singapura	*Vanda* Kapoho x *Arachnis breviscapa*	apical & axillary	slow	VWN	VWN, VWS	VWM	
27. Wee Huck Lay	*Arachnis* Ishbel x *Vanda lamellata*	apical & axillary	slow	VWN	VWN, VWS	VWM	
28. Wendy Scott 'Blue Bird'	*Arachnis hookerana* x *Vanda* Rothschildiana	apical & axillary	fast	VMW	VWN, VWS	VWM	
29. Wendy Scott 'Green Field'	*Arachnis hookerana* x *Vanda* Rothschildiana	leaf base & apical	slow	VWN (apical) MS+2-4-D+BA	VWN, VWS, MS	KCBP/ VWM	
30. Yvonne Tan	*Aranda* Queen of Purples x *Vanda* Rothschildiana	apical & axillary	fast	VWN	VWN, VWS	VWM	
Aranthera							
1. Anne Black	*Arachnis* Maggie Oei x *Renanthera coccinea*	apical & axillary	slow	VWN	VWN, VWS, MS	VWM	MS medium better than VWS for differentiation
2. Beatrice Ng 'Yellow'	*Renanthera storiei* x *Arachnis* Ishbel	axillary & leaf base	slow	VWN VWN+2-4-D +BA	VWN, VWS, MS VWN, VWS	VWM VWM	MS medium better than VWS for differentiation
3. Dainty	*Arachnis hookerana* var. *luteola* x *Renanthera monachica*	axillary	slow	VWN	VWN, VWS	VWM	

TABLE 2. LOCAL ORCHID HYBRIDS SUCESSFULLY MERICLONED

	Parentage	Source of meristem explant	Growth rate	Media for initiation (liquid)	Media for multiplication & differentiation	Media for plantlet formation	Remarks
Aranthera							
4. Francina Bruyns	*Aranthera* Dainty x *Renanthera storiei*	apical & axillary	slow	VWN	VWN, VWS	VWM	
5. Gracia Lewis	*Aranthera* Mohamed Haniff x *Renanthera storiei*	apical & axillary	slow	VWN	VWN, VWS	VWM	
6. James Storie	*Arachnis hookerana* x *Renanthera storiei*	apical & axillary	slow	VWN	VWN, VWS, MS	VWM	MS medium better than VWS for differentiation *Aranthera* spp. excrete strong phenolic compounds, need to change medium every few days during the first six months; when comparing them to *Vanda* and *Aranda*, their growth is extremely slow
Ascocenda							
1. Heah Hock Heng	*Vanda* Josephine van Brero x *Ascocenda* Hilo Rose	whole leaf	fast	MS+2-4-D +BA	VWN, VWS	VWM	
2. Botanic Gardens serial No. 4038	unregistered hybrid	apical & axillary	slow	VWN	VWN, VWS, MS	VWM	MS medium better than VWS medium for differentiation
3. Botanic Gardens serial No. 4766	unregistered hybrid	apical & axillary	slow	VWN	VWN, VWS	VWM	
Burkillara							
1. Ong Thye Chiew	*Aeridachnis* Bogor x *Vanda* Dawn Nishimura	apical & axillary	slow	VWN + NAA	VWN, VWS, MS	VWM	
Cattleya							
1. Meadii	*Cattleya bowringiana* x *Cattleya forbesii*	apical	slow	VWN	VWN, VWS	KCBP	root formation difficult, roots form in KCBP medium, but not in VWM medium
2. Botanic Gardens serial code C03	unregistered hybrid	leaf base apical	fast	MS+24D +BA	VWM, VWS	VWM	
Dendrobium							
1. Alice Spalding	*Dendrobium tokai* x *Dendrobium undulatum*	apical & axillary	fast	VWS/VWN	VWN, VWS	VWM	VWS solid medium good for all *Dendrobium* spp. for differentiation into shoot; rate of differentiation fast in this medium, but not good for *Vanda* spp.
2. Dendrobium Barbara Moore	*Dendrobium affine* x *Dendrobium gouldii* 'blue'	axillary	slow	VWS	VWN, VWS	VWM	
3 Caesar	*Dendrobium phalaenopsis* x *Dendrobium stratiotes*	apical & axillary	fast	VWS	VWN, VWS	VWM	
4 Cheong Chee Yon	*Dendrobium* Cheong Fook Sum x *Dendrobium* Queen Emma	apical	fast	VWN	VWN, VWS	VWM	
5. Ellen Harris	*Dendrobium phalaenopsis* x Ursula	apical & axillary	slow	VWN	VWN, VWS	VWM	
6. Fiery Gold	*Dendrobium* Ismail Trengganu x *Dendrobium* Guadalcanal	apical & axillary	slow	VWS	VWN, VWS	VWM	

TABLE 2. LOCAL ORCHID HYBRIDS SUCCESSFULLY MERICLONED

	Parentage	Source of meristem explant	Growth rate	Media for intiation (liquid)	Media for multiplication & differentiation	Media for plantlet formation	Remarks
Dendrobium							
7. Ng Eng Cheow	*Dendrobium* Alice Spalding x *Dendrobium* Jaquelyn Thomas	apical & axillary	fast	VWN	VWN, VWS	VWM	3 petals pinkish
8. Sunny	*Dendrobium* Beach Girl x *Dendrobium schulleri*	axillary	slow	VWS+ IAA	VWN, VWS	VWM	
9. Tay Swee Keng	*Dendrobium* Jaquelyn Concert x *Dendrobium* Irene Cheong	apical	fast	VWN	VWN, VWS	VWM	
10. Yong Kok Wah	*Dendrobium* Mary Trowse x *Dendrobium schulleri*	apical & axillary	slow	VWN	VWN, VWS	VWM	
11. Botanic Gardens serial No. 4879	unregistered hybrid	apical & axillary	fast	VWN	VWN, VWS	VWM	
12. Botanic Gardens serial No. 8090	unregistered hybrid	apical axillary	fast	VWS	VWN, VWS	VWM	
13. Botanic Gardens serial No. 8262	unregistered hybrid	axillary	fast	VWS + NAA	VWN, KCS, VWS	VWM	
14. Botanic Gardens serial No. 8324	unregistered hybrid	apical	fast	VWS	VWN, VWS	VWM	
15. Botanic Gardens serial No. 8961	unregistered hybrid	apical & axillary	fast	VWN	VWN, VWS	VWM	
16. Botanic Gardens serial No. 9215	unregistered hybrid	apical & axillary	fast	VWS+NAA	VWN, KCS, VWS	VWM	
17. Botanic Gardens serial No. 12917	unregistered hybrid	apical & axillary	slow	VWS	VWN, VWS	VWM	
Doritaenopsis							
1. Elizabeth Waldheim	*Doritis pulcherrima* x *Phalaenopsis* Lam Soon	lateral bud of flower stalk	slow	MS+2-4-D +BA	VWN, VWS	Tch	
2. Botanic Gardens serial PDI code 1	unregistered hybrid	lateral bud of flower stalk	slow	MS+24D +BA,VWS	VWN, VWS	Tch	
Holttumara							
1. Cochineal	*Aranda* Hilda Galistan x *Renanthera coccinea*	apical & axillary	slow	VWN	VWN, MS	VWM	
Kagawara							
1. Teoline Fair	*Ascocenda* Meda Arnold x *Renanthera storiei*	apical & axillary	slow	VWN	VWN, MS	VWM	
Mokara							
1. Sally Lim	*Arachnis hookerana* x *Ascocenda* Meda Arnold	axillary	fast	VWN	VWN, VWS, MS	VWM	
Oncidium							
1. Goldiana	*Oncidium flexuosum* x *Oncidium sphacelatum*	apical	fast	VWS	VWN, VWS	KCBP	Root formation slow, successful only in KCBP medium
2. Josephine	*Oncidium haematochilum* x *Oncidium lanceanum*	apical	slow	VWS	VWN, VWS	KCBP	

TABLE 2. LOCAL ORCHID HYBRIDS SUCCESSFULLY MERICLONED

	Parentage	Source of meristem explant	Growth rate	Media for initiation (liquid)	Media for multiplication & differentiation	Media for plantlet formation	Remarks
Phalaenopsis							
1. Botanic Gardens serial No. 3596	unregistered hybrid	flower stalk	slow	MS+2-4-D +BA	VWN, VWS	Tch	
Renanopsis							
1. Lena Rowold	*Renanthera storiei* x *Vandopsis laissochiloides*	apical & axillary	slow	VWN	VWN, MS	VWM	
Vanda							
1. Chia Kay Heng	*Vanda* Josephine van Brero x *Vanda* Dawn Nishimura	apical & axillary	fast	VWN	VWN	VWN	VWN solid medium good for protocorm multiplication and differentiation for *Vanda* spp.
2. Peopoe 'Diana'	*Vanda* Cooperi x *Vanda teres*						
3. Norbert Alphonso	*Vanda* Alice Laycock x *Vanda* Cooperi	apical & axillary	slow	VWN	VWN	VWM	
4. Patricia Low	*Vanda* Josephine van Brero x *Vanda* Jennie Hashimoto	apical & axillary	slow	VMW	VMW	VMW	
5. Ruby Prince	*Vanda* Ruby x *Vanda* Cooperi	apical & axillary	fast	VWN	VWN	VWM	
6. Sanada Kuma	*Vanda* B.P. Mok x *Vanda* Bill Sutton	apical & axillary	slow	VWN	VWN, MS	VWM	
7. Botanic Gardens serial No. 3102	unregistered hybrid	apical & axillary	slow	VWN	VWN	VWM	

Aranda Wendy Scott 'Green Field'

Aranda Freckles

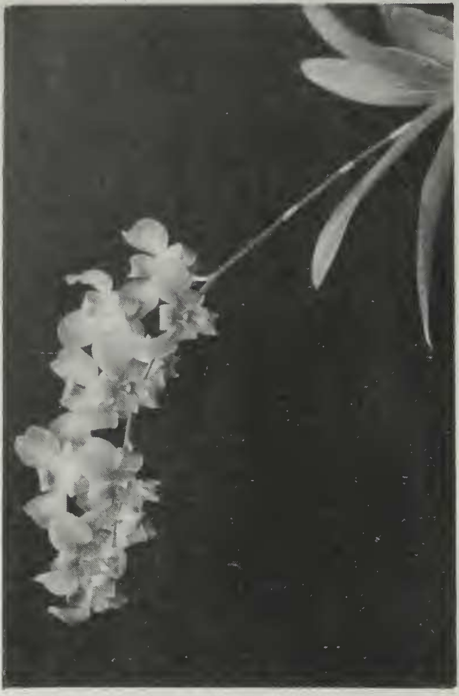
TABLE 3. NUTRIENT MEDIA COMPOSITIONS FOR TISSUE CULTURE OF LOCAL ORCHIDS (ALL VALUES IN UNITS OF MILLIGRAMMES PER LITRE)

	VWN (Liquid)	VWN (Solid)	VWS (Liquid)	VWS (Solid)	VWM (Solid)	KC (Liquid)	KCS (Solid)	KCBP (Solid)	MS (Solid)	MS+2-4-D+ BA (Liquid)	Tch (Solid)
$Ca_3(PO_4)_2$	200	200	200	200	200						200
KH_2PO_4	250	250	250	250	250	100	100	100	170	170	250
KNO_3	525	525	525	525	525				1900	1900	525
$MgSO_4$	250	250	250	250	250	100	100	100	370	370	250
NH_4SO_4	500	500	500	500	500	400	400	400			500
$MnSO_4$	7.5	7.5	7.5	7.5	7.5	0.1	0.1	0.1	16.9	16.9	7.5
$FeSO_4.7H_2O$	27.84	27.84	27.84	27.84	27.84	27.84	27.84	27.84	27.84	27.84	27.84
Na_2EDTA	37.24	37.24	37.24	37.24	37.24	37.24	37.24	37.24	37.24	37.24	37.24
$Ca(NO_3)_2.4H_2O$						400	400	400			
NH_4NO_3									1650	1650	
$ZnSO_4$						1.0	1.0	1.0	8.6	8.6	
$CuSO_4.5H_2O$						0.03	0.03	0.03	0.025	0.025	
$CaCl_2.2H_2O$									332	332	
KI						1	1	1	0.83	0.83	
$CoCl_2.6H_2O$									0.025	0.025	
$H_3B_2O_3$						1	1	1	6.2	6.2	
$Na_2MoO_4.2H_2O$									0.25	0.25	
$AlCl_3$						0.03	0.03	0.03			
Thiamine HCl									1	1	
Myoinositol									100	100	
Adenine sulfate									40	40	
Nicotinic acid									5	5	
IAA	0.1-0.5									0.3	
NAA	0.2										
6-amino-benzyladenine										0.5-2	
2- 4-D										1-2	
Coconut milk	150 ml	150 ml	150 ml	150 ml	75 ml	150 ml	150 ml		150 ml	150 ml	150 ml
Active charcoal											150
Tomato juice					75 ml						
Banana					75000			75000			
Pineapple juice								150			
Sucrose	–		1000-2000	1000-2000	2000		1000-2000	1000-2000	1000-2000	–	2000
Agar	–	800-1000	–	800-1000	1000	–	800-1000	800-1000	800-1000	–	800-1000
Distilled water	850 ml	850 ml	850 ml	850 ml	925 ml	850 ml	850 ml	850 ml	850 ml	850 ml	850 ml

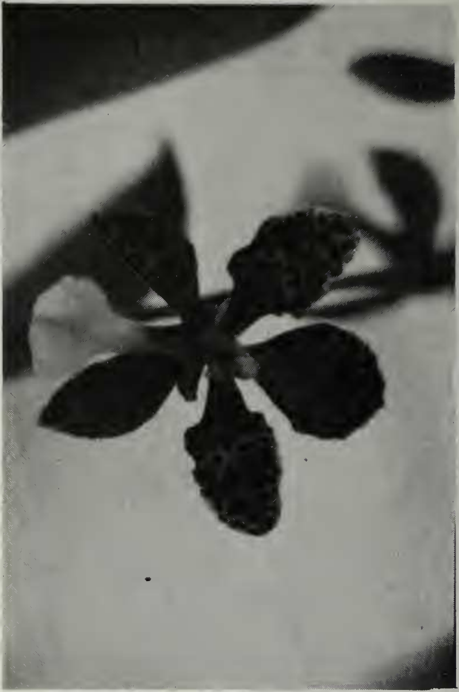
Vanda Sanada Kuma

Vanda Chia Kay Heng

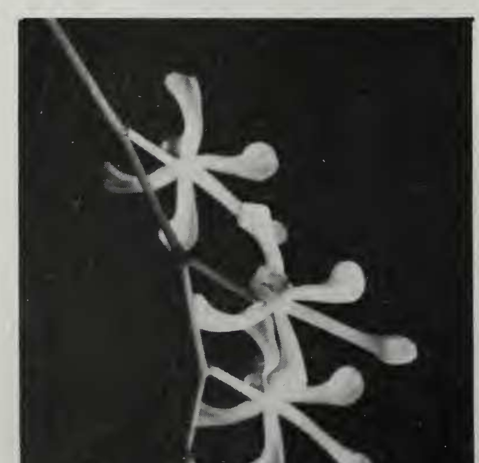
ORCHID SPECIES



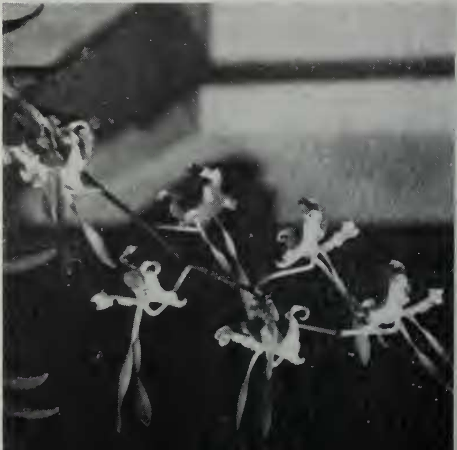
Dendrobium superbiens 'Superba'



Oncidium lanceanum



Arachnis hookerana



Dendrobium antennatum

ORCHID HYBRIDS

Dendrobium Botanic Gardens Serial No. 4879

Dendrobium Botanic Gardens Serial No. 9215

Dendrobium Cheong Chee Yon

Dendrobium Yong Kok Wah

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